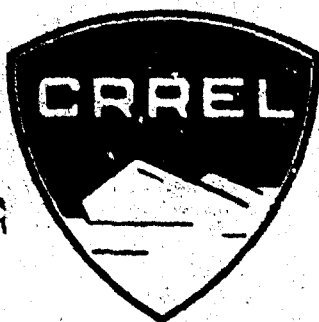


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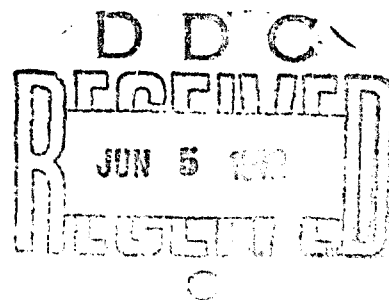
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THE MICROBIOLOGY OF TERRESTRIAL CRUDE OIL DEGRADATION

Patrick G. Hunt

April 1972



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13. ABSTRACT

It has been well established in the literature that microorganisms capable of decomposing crude oil are quite common in terrestrial areas around the world. It has also been well established that the straight chained hydrocarbons of C10 to C18 length, the kerosene fraction, are the most susceptible to microbial degradation. However, the shorter-chained hydrocarbons, the gasoline fraction, seems to be degraded more slowly, and in some instances may be toxic to microorganisms. The ring-structured hydrocarbons are also degraded but at a considerably slower rate than the straight chained hydrocarbons. The biochemical pathways by which hydrocarbons are degraded are not unique or few in number. There seems to be a substantial void in the literature concerning hydrocarbon decomposers in cold regions and the means of optimizing decomposition in cold terrestrial areas. It is possible, because of the long generation times of microbes in cold environments, that oil spills in cold terrestrial areas may be one of the few places where microbial inoculation may be beneficial. However, in such situations biological action is limited by environmental conditions and not by the presence of microorganisms.

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THE MICROBIOLOGY OF TERRESTRIAL CRUDE OIL DEGRADATION

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PREFACE

This paper was prepared by Captain Patrick G. Hunt, Research Soil Microbiologist, of the Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory.

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MICROBIOLOGY OF TERRESTRIAL CRUDE OIL DEGRADATION

by

P.G. Hunt

INTRODUCTION

The utilization of petroleum for products and services throughout the world has increased to monumental levels and large utilization increases are projected. However, the benefits that are realized from the use of petroleum products are somewhat negated by the increased probability of environmental damage from mishaps in the handling of petroleum. A particularly critical area of potential pollution is the transport of massive amounts of both crude and refined petroleum by ship and pipeline. Notable oil spill disasters such as the *Torrey Canyon* and Santa Barbara spills have already occurred, but considerable progress in prevention and clean-up materials and procedures has been made (personal observation at the 1971 Conference on Prevention and Control of Oil Spills).

However, since most oil mishaps have been on water most of this progress has been in the area of aquatic spills and relatively little has been done or considered in the area of terrestrial spills. Yet numerous petroleum transport systems are terrestrial. For example, the proposed Alyeska pipeline will cross 800 miles of ecologically sensitive terrain in Alaska. Thus the potential for massive ecological damage from terrestrial oil spills exists. Terrestrial oil spill clean-up is difficult in any area, but in Alaska, where permafrost soils and slow growing vegetation are prevalent, the potential problems are magnified immensely. In such a sensitive environment, physical and mechanical clean-up may cause more ecological damage than the spill itself. Therefore, after the potential water pollution and health hazards have been addressed, one of the most logical approaches for treating a terrestrial oil spill in Alaska is by microbiological means. This report concerns the topic of microbial decomposition of crude oil in soils.

CRUDE OIL

Crude oil is composed of numerous organic compounds that originate from direct or indirect biological action. The components can be classified as paraffins (alkanes), cycloparaffins (cycloalkanes or naphthenes), aromatics, asphalts, and combinations of these (Davis 1967). Other classifications of crude oil are also commonly used; Morrison and Boyd (1966) used distillation temperatures and carbon number to denote certain fractions of crude oil. The composition and characteristics of crude oil vary greatly with source, but compared to the extensive number of possible compounds and isomers the variation is relatively small (Meinschein 1959). The aromatics are normally found in lower quantities relative to the paraffins or cycloparaffins, and cyclopentane and cyclohexane often constitute large portions of the cycloparaffins. The three primary elements other than carbon of the asphaltic, non-hydrocarbon fractions are oxygen, sulfur and nitrogen; in some instances the asphaltic fraction composes as much as 50% of the crude oil (Davis 1967). The asphaltic crudes are often more toxic and less biodegradable than other types. However, spillage of any type of crude oil releases a large variety of organic compounds into the environment, and their removal with the least health and ecological damage is imperative.

CRUDE OIL DECOMPOSING ORGANISMS

Hydrocarbon-utilizing microbes are found from the tropics to the Arctic and have been isolated from soil, estuaries, animal feces, and numerous other locations (Miget et al. 1969, Brown and West 1970, Bushnell and Hass 1941). However, they are not omnipresent; Zobell (1969) reported their presence in the high seas to be practically nil. Bushnell and Hass (1941) demonstrated wide utilization of hydrocarbons by the genera *Pseudomonas* and *Mycobacterium*; they also cited Schryver (1906), Kaserer (1906), Störmer (1908), and Tatum and Peter (1919) for early observations of hydrocarbon decomposing organisms. Fuhs (1961) listed over 100 organisms capable of decomposing hydrocarbons. *Streptomyces*, *Nocardia*, *Mycobacterium*, *Corynebacterium* and *Brevibacterium* were cited as the most commonly isolated hydrocarbon decomposers by Foster (1962). Even though the number of isolated hydrocarbon decomposers reported is extensive, they were generally isolated by plating, and plating is considered to isolate only 1 to 10% of the organisms present in a plated soil (Casida 1968). Foster (1962), for instance, cited some organisms that could not grow on hydrocarbon media but could grow on the more selective media that utilized N-tridecane as the single carbon source. This serves to illustrate that numerous organisms in the environment can degrade oil at very slow rates, but their isolation and rate of oil decomposition depend upon the selection conditions to include competition with other organisms for carbon sources, nutrients and moisture. In addition to those organisms that grow on hydrocarbons, some organisms oxidize hydrocarbons but do not utilize them for growth (Leadbetter and Foster 1959, 1960, Davis and Raymond 1961). Thus, when the large number of hydrocarbon utilizers and oxidizers is considered, it becomes apparent that even though some organisms can decompose certain fractions of crude oil much more readily than others, the decomposition of crude oil in the soil is carried out by numerous organisms rather than by any particular species or, as will be shown, by any particular biochemical pathway.

BIODEGRADABILITY OF VARIOUS CRUDE OIL FRACTIONS

Zobell (1969) stated that more organisms were capable of decomposing C10 to C18 (kerosenes) than any other fraction. Similar findings have been reported by Kator et al. (1970). This pattern of degradation would be intuitively expected since these compounds are not as volatile as the shorter chained hydrocarbons nor as sterically complicated as the larger or branched hydrocarbons. Davis (1967) stated that the gaseous hydrocarbons of low molecular weight, methane through n-butane, were oxidized at a rate usually limited by the solubility of the gas in the aqueous surroundings of microbial cells. The gaseous phase of 5-10 carbon hydrocarbons was also reported to be utilized most readily. However, the liquid phase of these low molecular weight hydrocarbons (paraffins and cycloparaffins) was found to retard microbial growth and in some cases be refractory. Similarly, Davis and Raymond (1961) found that aromatic hydrocarbons of this size were often toxic to growth of *Nocardia*, but the toxicity was mitigated by the presence of other degradable hydrocarbons. This supports the view that the organisms in a natural system such as the soil of an oil spill area would be less affected by toxic components of crude oil than organisms exposed to a medium containing a single toxic component of crude oil as the carbon source. McKenna and Kallio (1965) demonstrated the variation of biodegradability of hydrocarbons with molecular structure, using growth of species of *Micrococcus*, *Pseudomonas*, *Mycobacterium* and *Nocardia* as their criteria of degradation. Even one methyl group on a straight chained hydrocarbon reduced its utilization, and dimethyl or especially neopentyl groups greatly reduced microbial utilization. Alkanes with terminal phenyl groups were utilized more readily than alkanes with phenyl substitution along the carbon chain.

Davis (1967) reported that extracts of oil from soil under both field and laboratory conditions were characterized by high absorption of infrared at 12.35μ and 13.4μ , indicating large percentages of aromatic structures. He also found that even after 45-62% by weight of crude oil was decomposed there was very little change in the oil composition except the paraffins. The slow degradation of the aromatics was also indicated by Zobeil (1960) who found that polycyclic hydrocarbons as well as paraffins were oxidized by bacteria more readily than was benzene. These studies indicate that the highly volatile and straight chained fractions of crude oil are likely to be removed from an oil spill area in a few weeks or months under ideal conditions. Their removal under poor degradation conditions will still be more rapid than the highly branched, asphaltic and aromatic type compounds which are likely to remain in an oil spill area for extended periods even under ideal conditions.

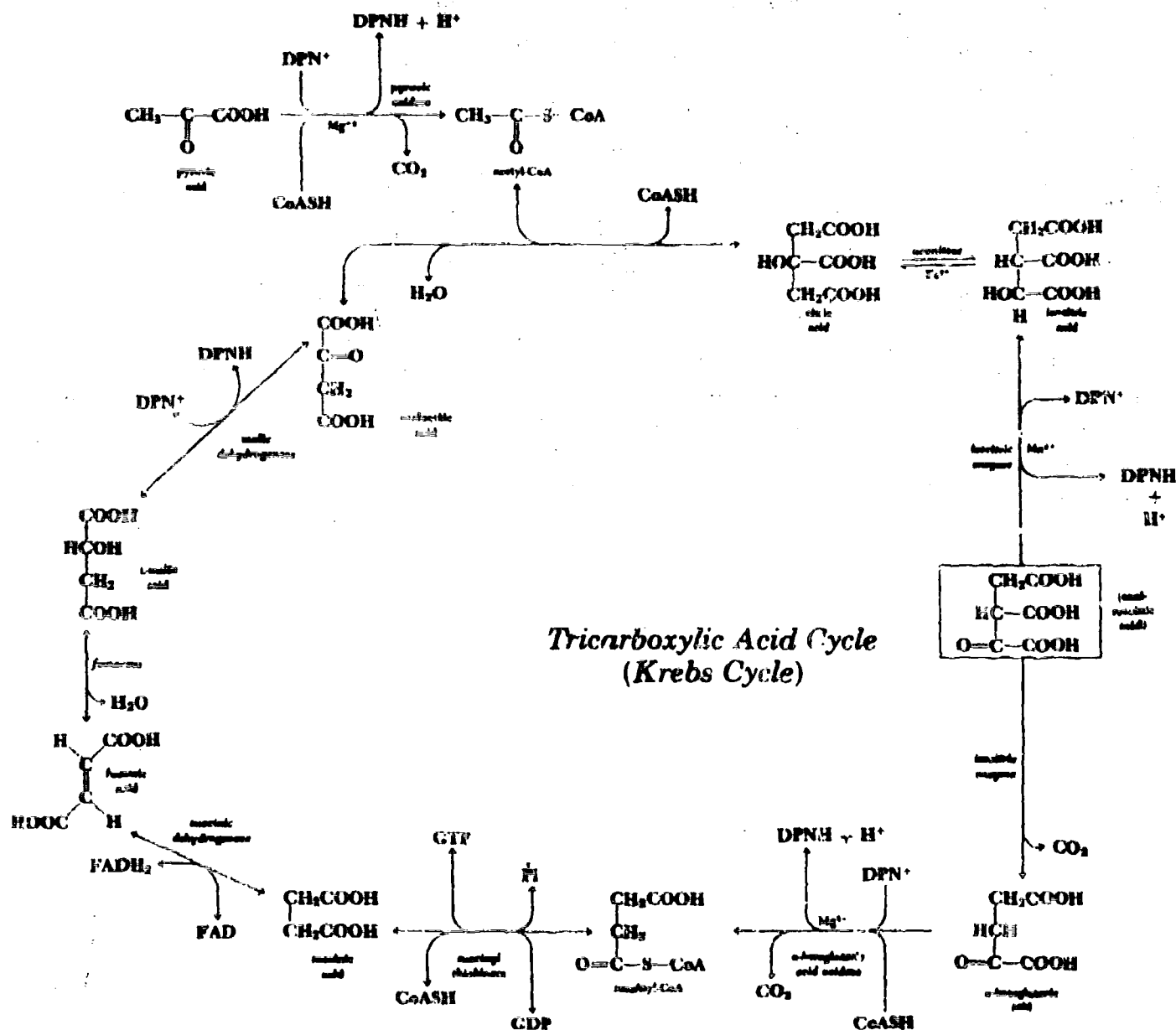
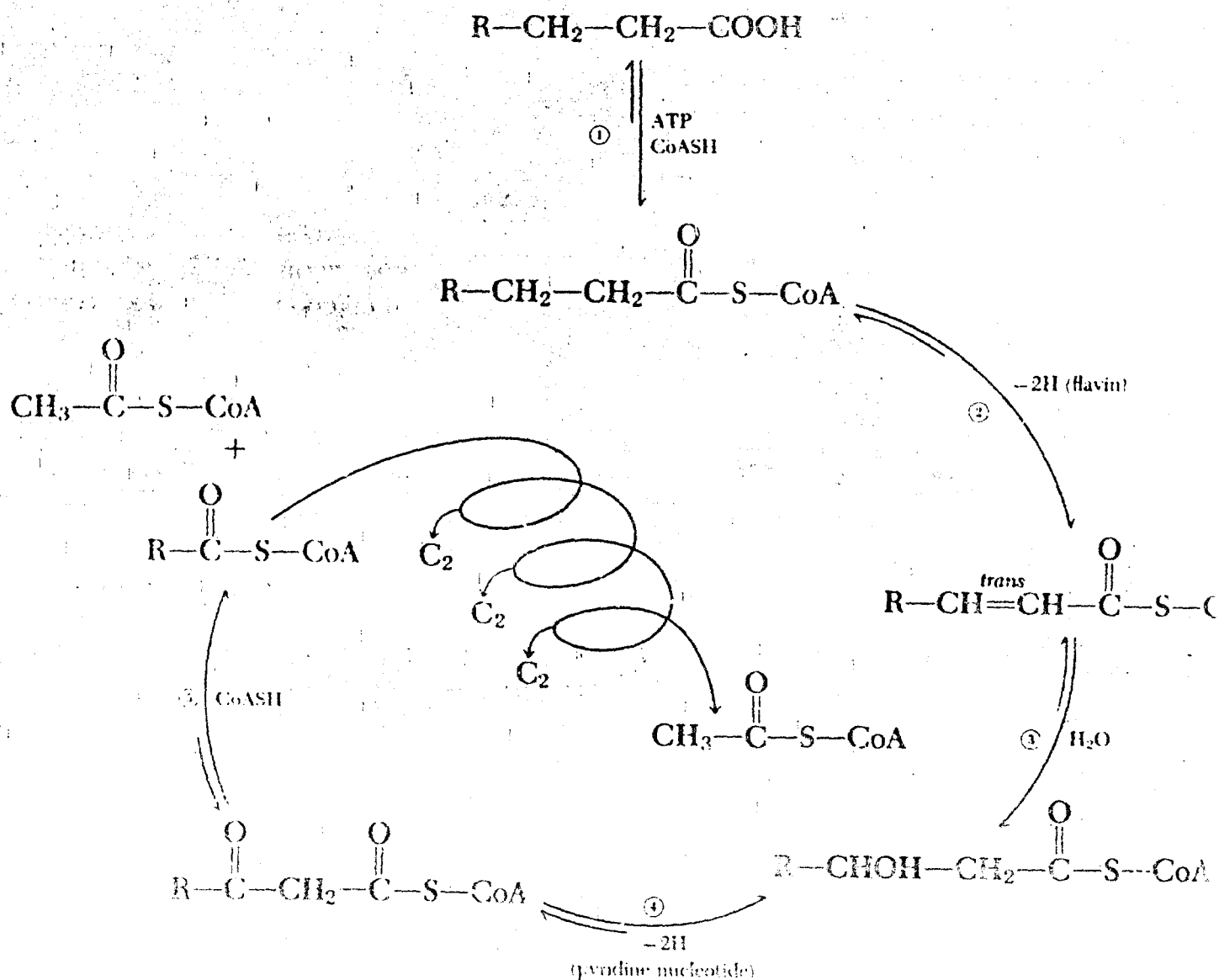


Figure 1. A schematic of the TCA cycle (Conn and Stumpf 1966).

BIOCHEMICAL PATHWAYS OF HYDROCARBON DECOMPOSITION

The tricarboxylic acid (TCA) cycle and the beta oxidative pathway (Fig. 1, 2) will be discussed in general terms to help the reader who is not biologically oriented understand how the degradation products of hydrocarbon are converted to energy, metabolites, CO_2 , and water. However, neither the precise biochemistry of these processes nor their connection to various molecular metabolites are addressed. These topics are discussed ably by Mahler and Cordes (1966), White, Handler and Smith (1968), and Conn and Stumpf (1968). The reactions that are presented are not intended to be a complete list of the hydrocarbon degradative pathways, but they are examples of how several fractions of crude oil can be degraded.



① Fatty acid thiokinases

② Fatty acyl-CoA dehydrogenases

③ Enol hydratase

④ β -Hydroxyacyl dehydrogenase

⑤ β -Ketoacyl thiolase

In a review of the biology of hydrocarbons, McKenna and Kallio (1965) cited several organisms that produced alcohols, acids, ethers and ketones in their decomposition of saturated aliphatic hydrocarbons (paraffins). They presented three major pathways of degradation (Fig. 3). In these reactions the only thing that separates the hydrocarbon from typical fatty acids of the various oxidative processes is the addition of a carboxyl group to one of the terminal carbons, an addition that can be brought about by relatively simple biochemical oxygenations and hydroxylation. As shown in Figure 2, the acid can readily be degraded through β -oxidation to acetyl CO-A an acid of two less carbon length, and energy in the form of reduced pyridine nucleotides and flavin coenzymes. In addition, the acetyl CO-A can enter the TCA cycle and be decomposed to CO_2 , water and energy or channelled into numerous biochemical processes. Thus, the simple biochemistry of the degradative pathways of paraffins is consistent with their relatively rapid decomposition in oil spill areas.

McKenna and Kallio (1965) also cited research that demonstrated that *Candida*, a yeast, could degrade alkenes by forming diols from the double bond, but bacteria preferentially oxidized the saturated end of alkenes (Stewart et al. 1960). *Pseudomonas* has been shown to degrade alkenes by formation of epoxides at the double bond. These general pathways are presented in Figure 4. Again in the case of alkenes the biochemical steps required for these compounds to enter the common oxidative pathways are simple.

The cycloparaffins and aromatic compounds have been shown to be much less degradable than the alkanes and alkenes, but they are degraded. Often the degradable side chains of a ring compound will be degraded before the ring is cleaved. Both cycloparaffin and aromatic rings tend to be degraded via the formation of diols and diacids (Elliot et al. 1959), and depending upon where the aromatic ring breaks a diacid or semialdehyde is formed (Fig. 5). Some cycloparaffins can be converted to a common cellular constituent by relatively simple steps such as decalin to adipic acid or pimelic acid (Fig. 6). Adipic acid can be shunted to the TCA cycle as succinic acid by an oxygenation and the removal of a terminal acetyl group as acetyl CO-A. Pimelic acid is involved in biotin synthesis in some microorganisms and its diamine form is a cell wall component of many bacteria.

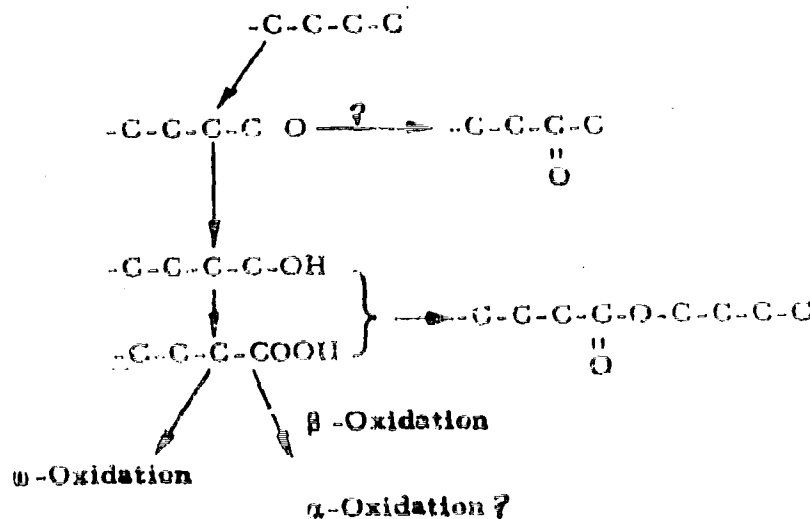


Figure 3. A schematic of saturated aliphatic decomposition from McKenna and Kallio (1995).

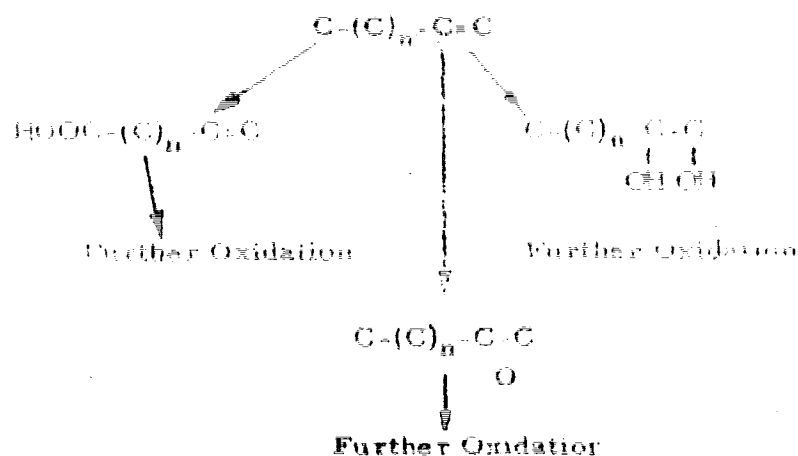


Figure 4. A schematic of alkene decomposition from McKenna and Kallio (1965).

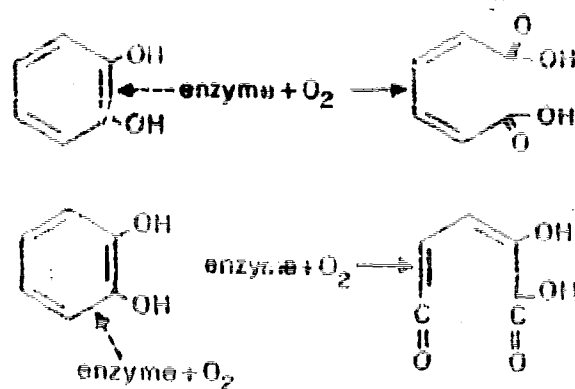


Figure 5. A schematic of aromatic ring cleavage (dashed arrows indicate point of enzyme cleavage).

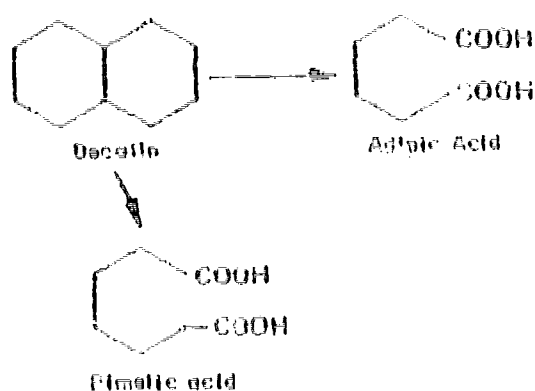


Figure 6. A schematic of decalin degradation (McKenna and Kallio 1965).

The more complex ring compounds are sterically more complicated and their degradation requires more complicated enzymes than those required for paraffin. However, even for this group of compounds a degradative pathway that is relatively simple can be envisioned. Figure 7 shows a schematic for phenanthrene and anthracene degradation to catechol. This degradative scheme is even more enlightening when it is realized that many organisms readily convert catechol to succinate and acetyl CO-A (Fig. 8) (Lamanna and Malette 1965).

The asphaltic fractions of crude oil are degraded by similar pathways, but their degradation involves desulfation, diamonization or some similar step that adds another restrictive factor in their degradation. Thus it can be seen that the degradation of crude oil components occurs via many very common biochemical pathways. However, the reactions can easily be limited by one slow step or environmental condition. Seldom are the conditions in soils such that an organism which grows on one of the more complicated components of crude oil will be able to compete favorably with those organisms that grow on relatively simple compounds. Therefore, actions that change the limiting growth factors of hydrocarbon decomposers to optimum levels will probably be the most effective means of increasing the rate of microbial degradation of crude oil in spill areas.

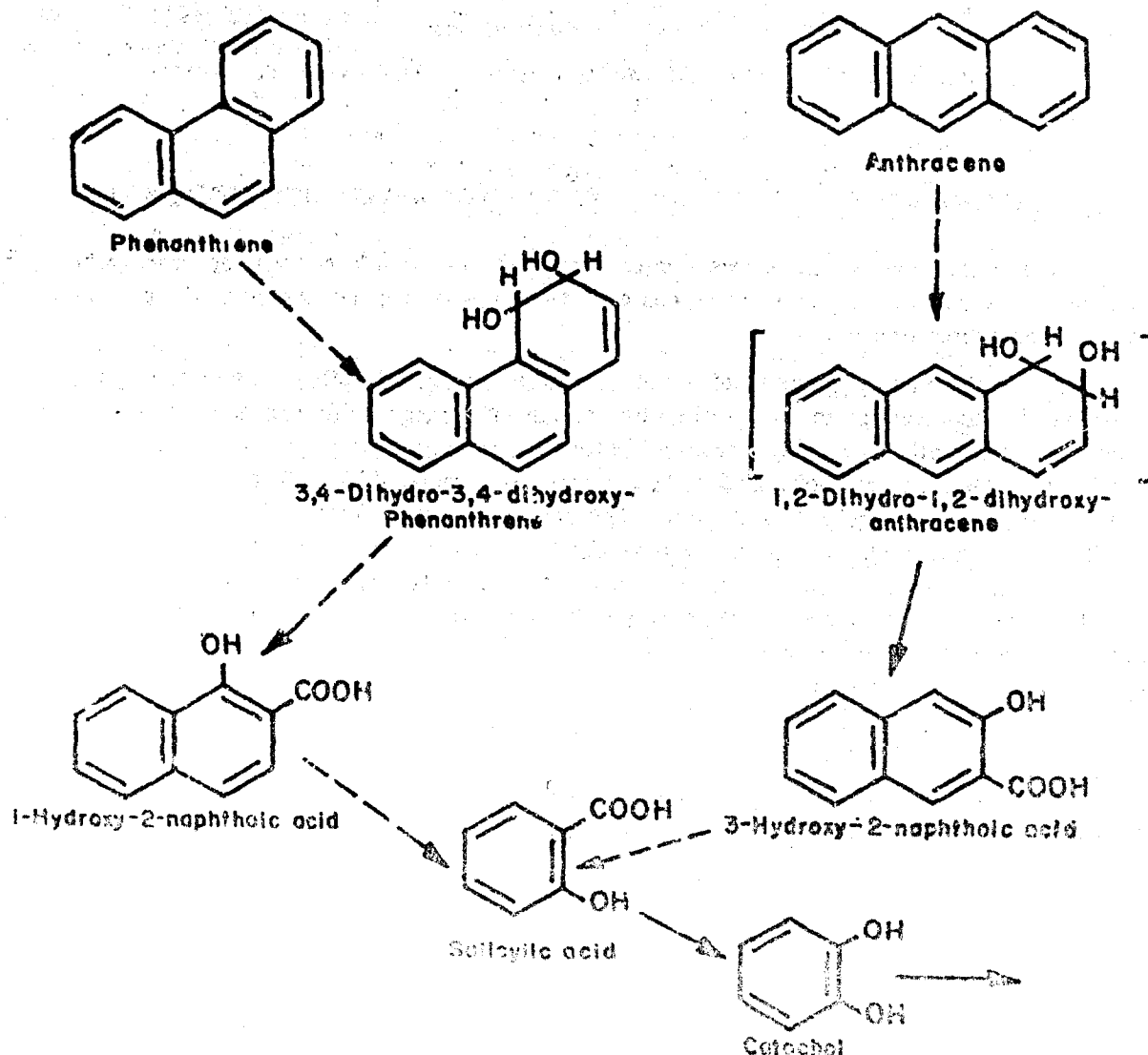


Figure 7. A schematic of phenanthrene and anthracene degradation to catechol (McKenna and Kallio 1965).

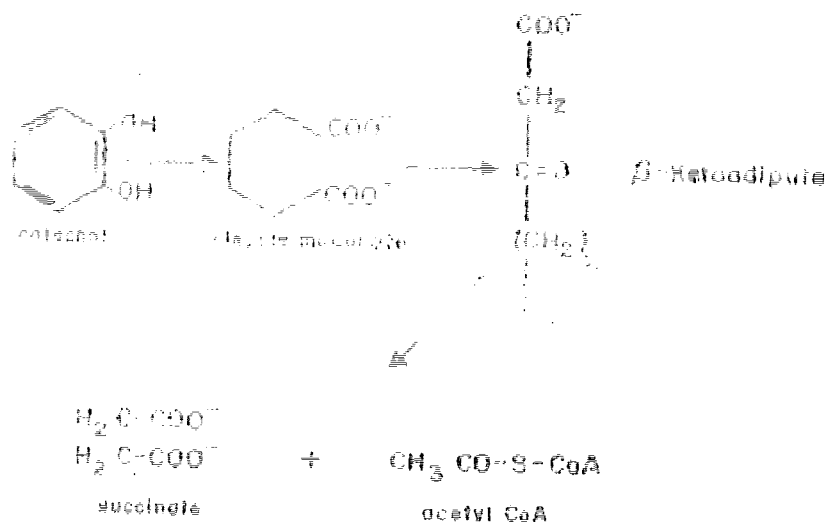


Figure 8. A schematic of catechol coconversion to TCA cycle constituents.

ENVIRONMENTAL CONDITIONS AFFECTING CRUDE OIL DECOMPOSITION

As previously discussed, numerous microorganisms are capable of utilizing hydrocarbons as their source of carbon. However, numerous conditions speed, impede or change the pathways of hydrocarbon decomposition.

Possibly the most pronounced effect of a large terrestrial oil spill on a microbial population is the sudden presence of an almost unlimited amount of decomposable carbon. Bacteria tend to decompose crude oil components more readily than the fungi or actinomycetes, but they are generally less efficient utilizers of added carbon material (Alexander 1981). They excrete large percentages of acids, alcohols, ketones, etc. The carbon of crude oil may quickly travel many different biochemical routes such as being evolved as CO_2 , secreted or excreted as a cell metabolite, or assimilated, or incorporated into cellular material. Numerous fungi, actinomycetes and bacteria are able to utilize the secretions and excretions of bacteria that attack the more complex or biodegradable natural organic macromolecules to the soil (Alexander 1981). Thus, a large population of non-hydrocarbon decomposers will accompany the increase in the hydrocarbon-decomposing population.

For a microbial population to expand rapidly it must be able to produce sufficient protein. When the source of oxidizable carbon is almost unlimited, as in an oil spill area, the limiting factor in protein production is often the availability of sufficient nutrients, especially nitrogen and phosphorus. If nitrogen or phosphorus are marginally available the population of hydrocarbon decomposers will be in competition with the utilizers of the decomposition products. The two types of populations are then likely to increase and decline in a cyclic fashion with one type's growth limited by lack of decomposition products and the other's by lack of nutrients. Therefore, one of the most obvious ways of increasing the rate of crude oil decomposition is to optimize the concentrations of nutrients, particularly nitrogen and phosphorus.

The physiology of many organisms, both anaerobic and aerobic, is pH sensitive. Nitrogen is only highly available in low pH soils. A pH slightly above neutral is best for nitrification and the subsequent availability of nitrogen to decomposing organisms. In addition to the biological factors, the solubility and availability of phosphorus, which is usually bound into insoluble iron

and aluminum *oxides* below pH 6 and calcium forms above pH 8, is highest within the pH range of 6-8. Soil pH can be adjusted with a number of products, but either sulfur or lime is commonly used. The adjustment of soil pH should be one of the first steps in the biological recovery of an oil spill area (Patrick Hunt, unpublished data).

Possibly the most important but most difficult to control environmental condition in crude oil degradation is the presence of sufficient oxygen to ensure aerobic conditions. Organisms carrying out aerobic decomposition of organic materials added to the soil typically convert 20 to 40% of the utilized carbon to cellular material, but anaerobic decomposers usually are only able to convert 10 to 20% of the utilized carbon to cellular material (Alexander 1964). Thus, when a anaerobic environment is created in the soil, it is not self-correcting. Anaerobic conditions are generally thought of in relation to waterlogged soil, but anaerobiosis can be created by a rapid consumption of oxygen. In many instances where large amounts of oxygen are consumed during decomposition of an added organic material, oxygen consumption will exceed the diffusion rate of oxygen, and the soil environment will become anaerobic. Then the microbial population will oscillate in response to the aerobic and anaerobic conditions. Under these conditions, those organisms that are able to decompose crude oil under both aerobic and anaerobic conditions, facultative organisms, have a distinct advantage.

Since oxygen diffuses through oil more rapidly than water, oil spills that occur on water-saturated or highly moist soils have lower rates of oxygen diffusion and consequently slower microbial decomposition. In addition work by McCown, Brown and Murrmann (1971) indicates that a spill on a waterlogged soil would cover more surface area because of slower oil penetration into the soil profile. In this type of situation microbial degradation would be exceedingly slow, and the possibility for extensive water pollution and health hazards exists. Thus, mechanical means of oil spill recovery and abatement may be required.

Temperature has a pronounced effect on both chemical and biological reactions, and the low temperatures of Alaska have an impeding effect on the rate of oil spill recovery by microbial means. At temperatures near the minimum for an organism metabolism, the generation time is often increased as much as 25 to 30 times (Lamanna and Mallette 1965). This is probably why oil degrading microorganisms have been observed to increase the rate of degradation of Prudhoe crude at 15°C (Patrick Hunt, unpublished data). The exact mode of microbial inhibition by low temperatures is not known, but most organisms reach their minimal temperature before their protoplasm ceases to exist in a free state.

However, some organisms, the psychrophiles, grow best at temperatures between 0 and 20°C, and numerous organisms such as algae in the North Sea (Robert Benoit, personal communication) and fungi in USA CRREL's permafrost tunnel (personal observation) are known to carry on metabolism below 0°C. In addition genetic selection for those organisms that can carry on substantial metabolism at low temperatures should have occurred in Alaska. Intuitively, it would seem that those organisms that could carry on substantial metabolism at low temperatures but increase their metabolism during the warmer Alaskan summer would survive better than the true psychrophiles, but a substantial void exists in the literature concerning microbial decomposition of hydrocarbons under cold conditions. The effect of temperature on hydrocarbon decomposition is further complicated by the fact that many reaction rates are dependent on vapor pressure and vapor pressure decreases with temperature (Davis 1967).

The interaction of many organisms, environmental conditions and human responses has been shown to be involved in crude oil degradation and incorporation into soil organic material and the precise processes that will be most important at any one time are not explicitly clear. However, one thing is clear: in a cold environment such as Alaska, the natural recovery of an oil spill will be slow and may take more than 15 to 20 years (Rickard and Doncho 1971), but it is quite likely to result in the least total environmental damage.

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